Effect of targeted ovarian cancer therapy using amniotic fluid mesenchymal stem cells transfected with enhanced green fluorescent protein-human interleukin-2 in vivo

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Agenda Style

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02 Material and method

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Introduction

Introduction

Ovarian cancer is one of the most let hal types of gynecological malignanc

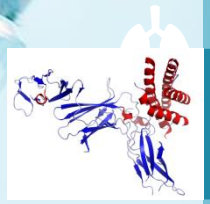
chemotherapy is poor in recurrent pati ents[1]

aim of current research is to establi sh novel therapeutic methods

Cell lines and animal models are valu able research tools[2]

In our previous studies, the SKOV3 h uman ovarian carcinoma cell line[3]

introduction

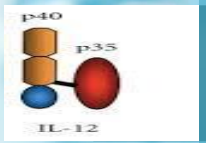


IL-2

cytocine

Function[4,5]

- **stimulating the proliferation of T cells**
- **inducing cytotoxic T lymphocyte generation**
- **inducing the production of natural killer cells**



Increase[6]

In cancer, including ovarian cancer, metastatic melanoma and renal cell carcinoma

Introduction

- when IL-2 is administered systemically at high doses have side effect:

- malaise, fever, nausea, and vomiting[7]

.

- more severe reactions, hepatic dysfunction, increased capillary permeability and decreased systemic vascular resistance[8]

- therapeutic strategy that specifically delivers IL-2 to the tumor location may significantly reduce the required IL-2 dosage

introduction

stem cells are becoming an im portant source of cells for cellul ar therapy[9]

mesenchymal stem cells is olated from human second a nd third trimester AF and

IL-2 and the green fluorescent prot ein (GFP) gene fused form a plas mid vector,transfect to MSC

intravenously injected at various doses i nto ovarian cancer nude mice

Tumor formation, the expression hIL-2 were analyzed

The aim of the study evaluate the migrato ry AF-MSCs into tumor cells in vivo, deter mine their function as delivery vehicles for anti-tumor molecules, such as IL-2

Material method and Result

Isolation and culture of MSCs

- ethical approval.
- consent was obtained from each volunteer
- AF samples from 30 female volunteers
- The samples centrifuged at 100 g for 5 min
- DMEM,FBS,Penestrep
- non-adhering cells were removed.

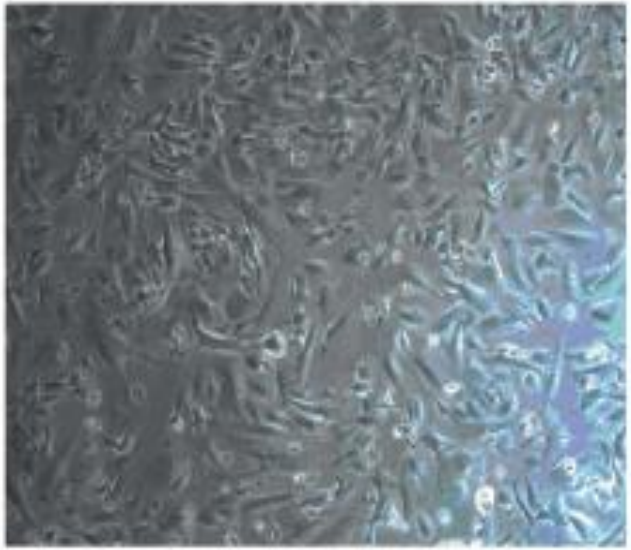
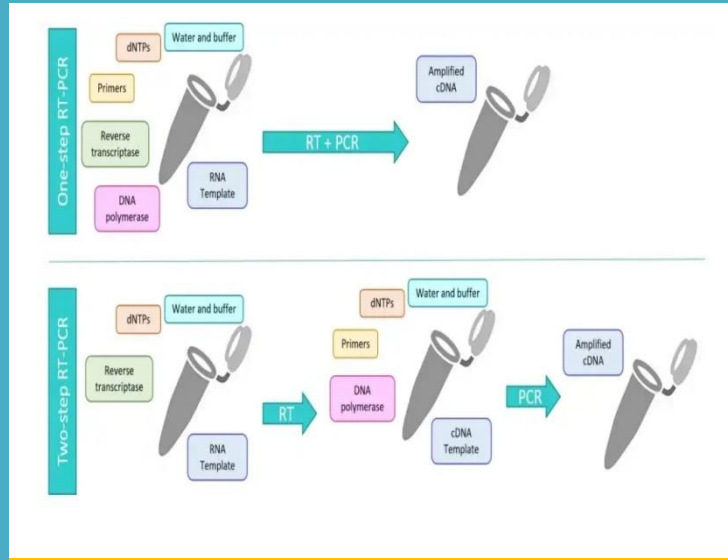


Figure 3. Human AF mesenchymal stem cells from second trimester AF (magnification, x50). AF, amniotic fluid.

characterization Msc

- Markers of AF-MSCs analysied by RT-PCR
- Total RNA extracted from MSCs using Tri Reagent,onestep pcr
- Primers oct 4
  - sence:5'-CGTGAAGCTGGAGAAGGAGAAGCTG-3'
  - anti sense:5'-CAAGGGCCGCAGCTTACACATGTTC-3‘
- Primers B-actine
  - sense: 5’;TGGCACCACACCTTCTACAATGAGC-3’
  - anti sense:5'-GCACAGCTTCTCCTTAATGTCACGC-3'



2)Tri reagent:guanidinium thiocyanate-phenol-chloroform

characterization Msc

- NTERA-2 cells line a pluripotent embry onic carcinoma as positve controll
- MRC-5 cells line a human diploid fibrobl asts lung tissue as negative controls

1)Oct4 are transcription factors It is crit ically involved in the self-renewal of un differentiated stem cells

2)Highly expressed in SC,emberyonic c arcinoma cell,emberyonic germ cell.

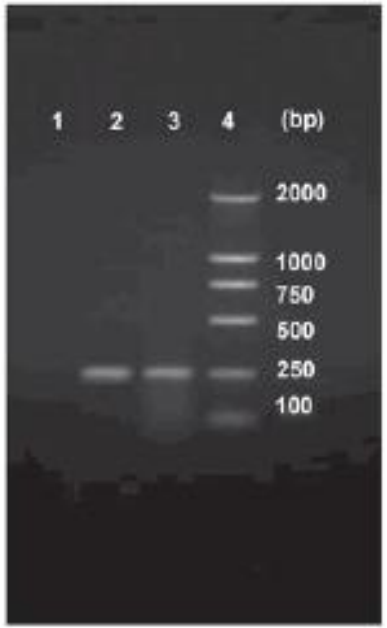


Figure 5. Enhanced green fluorescent protein-transfected amniotic fluid mesenchymal stem cells express OCT4. Lane 1, negative control; lane 2, positive control; lane 3, OCT4 mRNA; lane 4, marker. OCT4, octamer-binding protein 4.

EGFP gene transfection into MSCs

- 40-micro l Lipofectamine 20 h mixe d with AF_MSC
- One week later, the stable transfec tants were selected with G418
- expression of EGFP in the cell mo nitored under UV microscope

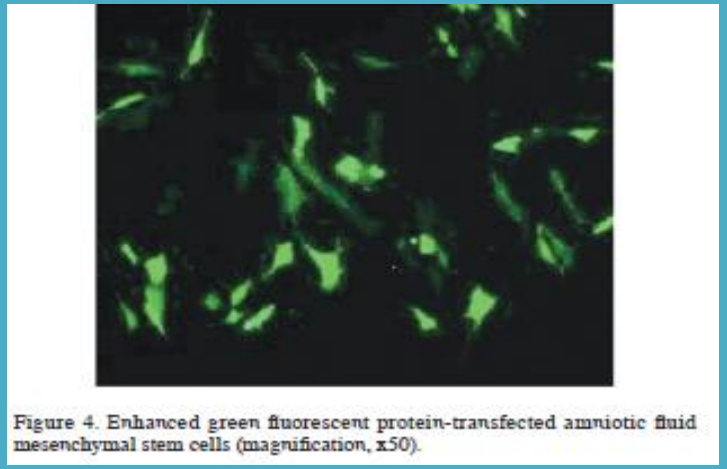
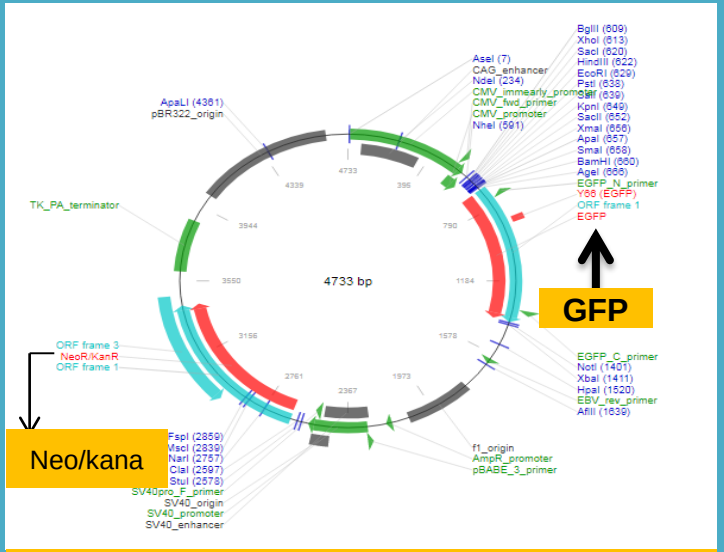


Figure 4. Enhanced green fluorescent protein-transfected amniotic fluid mesenchymal stem cells (magnification, x50).



G418 blocks polypeptide synthesis by in hibiting the elongation step in both proka ryotic and eukaryotic.

hIL-2 gene extraction

- Peripheral blood samples
- lymphocyte separating medium c entrifuged at 150 x g for 15 min
- First layer, plasma; second layer, lymphocytes and monocytes; thir d layer, lymphocyte
- broken down using TRIzol.Rna e xtract. RT-PCR was performed t o synthesize cDNA(462 bp)

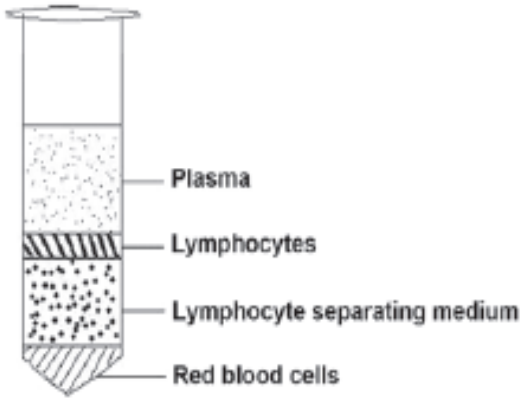


Figure 1. Blood was separated into four layers using lymphocyte separating medium.

Upstreamprimer:5'GGAATTCATGTACAGGATG3‘

Downstream primer:5'GACTGAACTCAGCTGG3'

hIL-2 gene identification

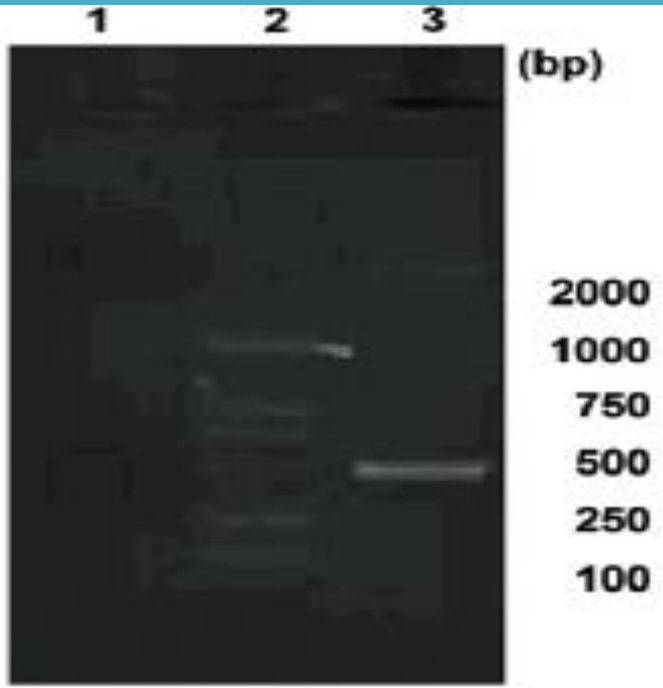


Figure 7. Reverse transcription polymerase chain reaction analysis of hIL-2 gene expressed. Lanes: 1, water negative control; 2, marker; 3, hIL-2. hIL-2, human interleukin-2.

hIL-2 gene segment cloning and identification.

- product was combined with a pMD18-T Simple Vector
- JM109 competent cells,heat shoke
- spread on (LB) agar plates wi th X-gal, digested with EcoR I and Sal I , electrophoresis was performed

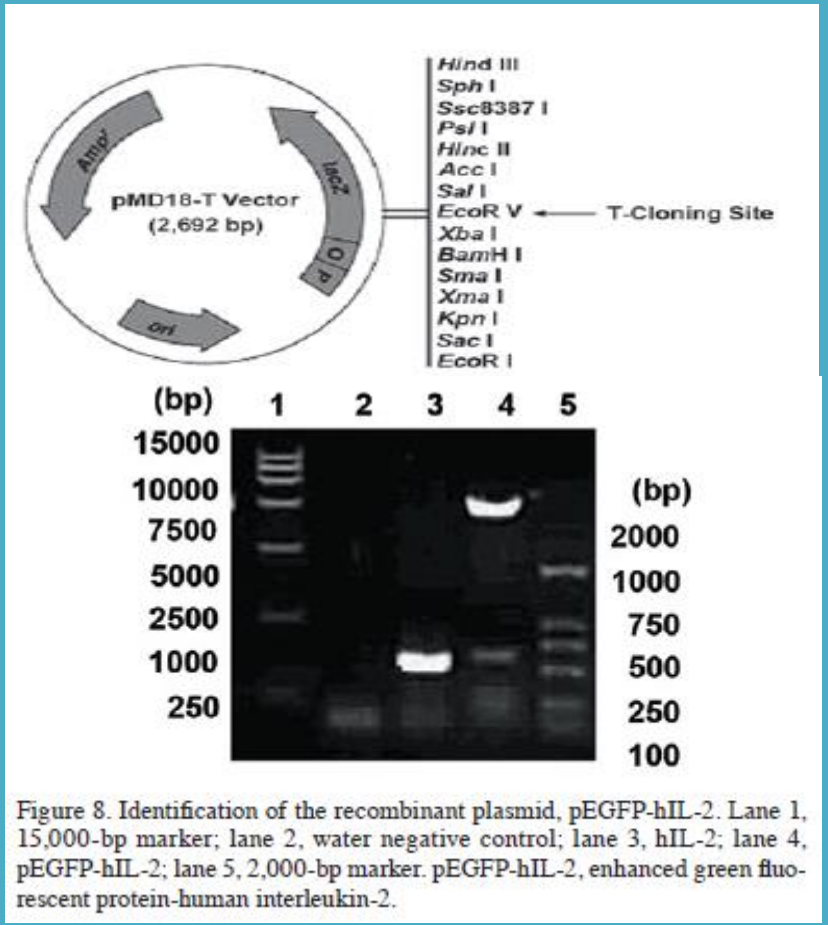
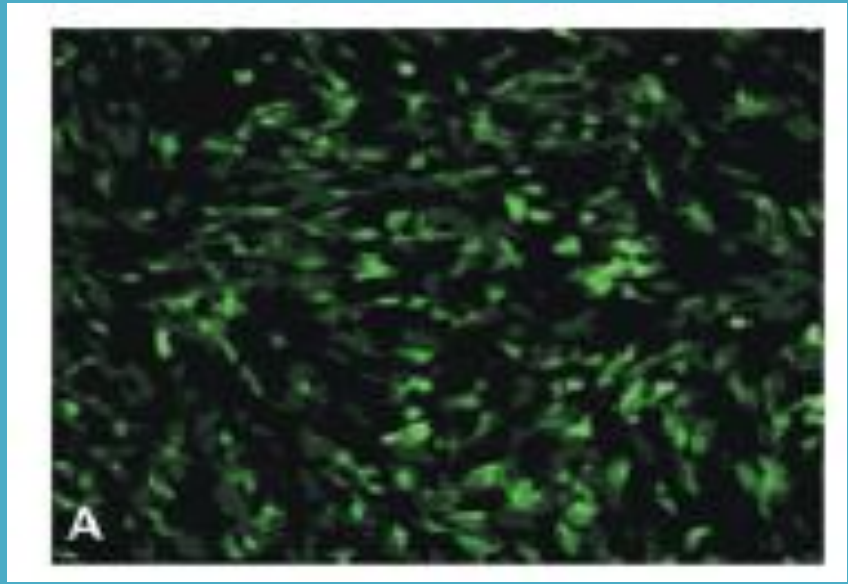


Figure 8. Identification of the recombinant plasmid, pEGFP-hIL-2. Lane 1, 15,000-bp marker; lane 2, water negative control; lane 3, hIL-2; lane 4, pEGFP-hIL-2; lane 5, 2,000-bp marker. pEGFP-hIL-2, enhanced green fluorescent protein-human interleukin-2.

transfection of pMD-hIL-2 into AF-MSCs

- AF-MSCs were inoculated during the logarithmic growth phase
- pMD-hIL-2 was transfected into the A F-MSCs using Lipofectamine
- the cells were selected using 500 μg/ ml G418
- hIL-2 detection kit, GFP gene expres sion detected fluorescence microsco pe



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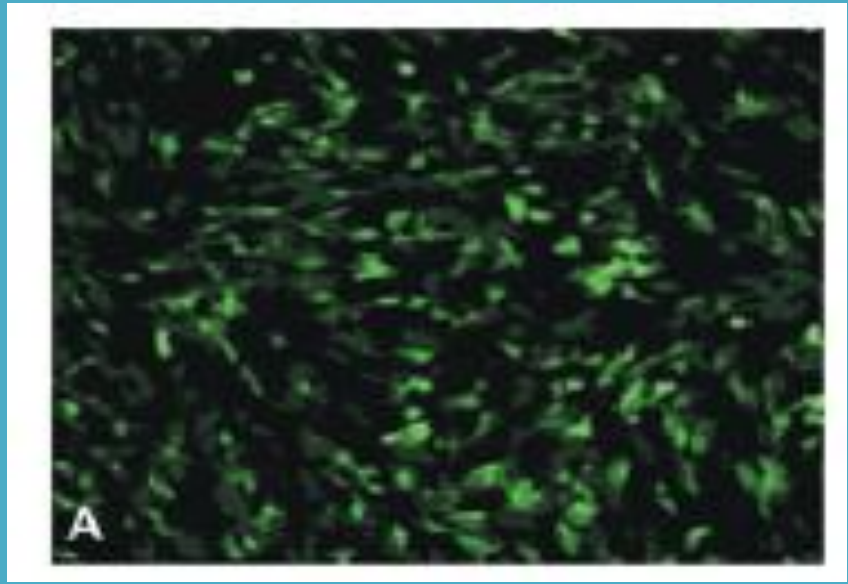

experssion il-2

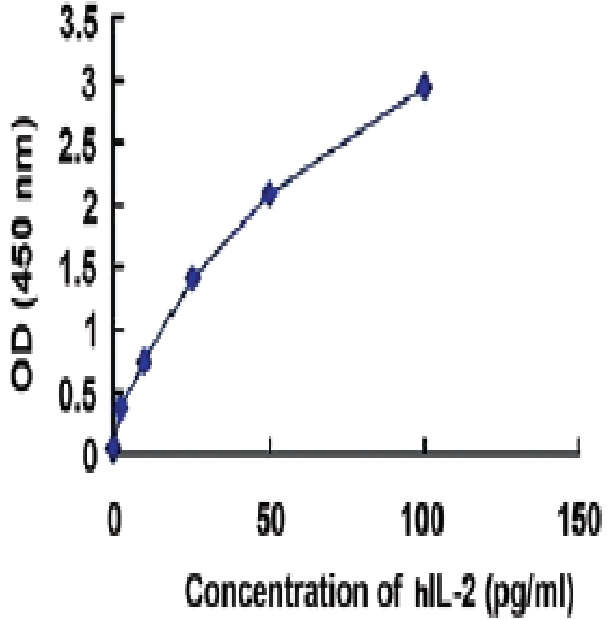

Figure 10. Expression of the hIL-2 gene following transfection into amniotic fluid mesenchymal stem cells. OD, optical density; hIL-2, human interleukin-2.

Establishing an ovarian cancer animal

- SKOV3 ovarian cancer cells
- nu/nu-BALB/c nude mouse
- subcutaneously injected into the scapula region.
- 2 or 18 days before tumor cells inoculation
- tumor reached 1 cm in diameter
- AF-MSCs transiently transfected, pMD-hIL-2 were injected the caudal vein of each ovarian cancer mouse
- Six weeks green fluorescence was apparent around the tumor tissue

Establishing an ovarian cancer animal

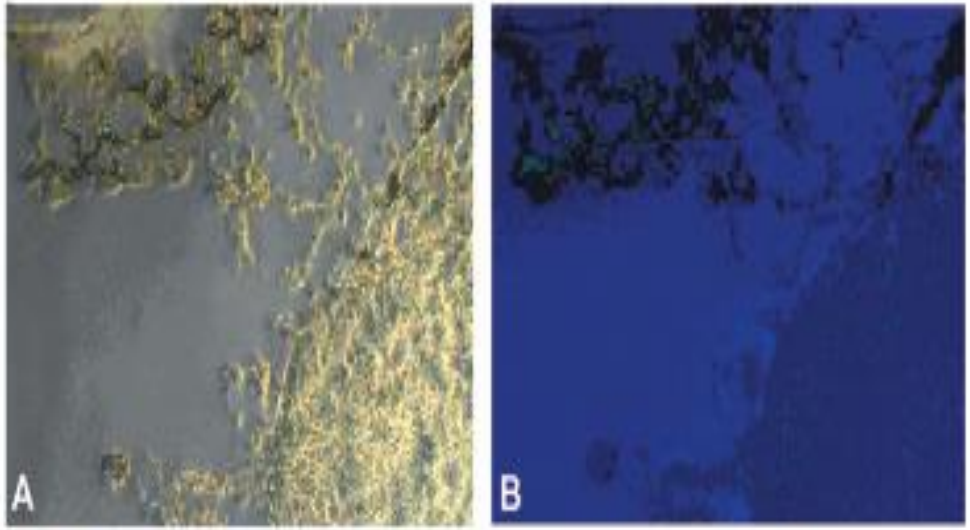

Figure 12. GFP-labeled amniotic fluid mesenchymal stem cells surrounding the tumor mass. The GFP-specific signal as observed by (A) phase contrast microscopy (magnification, x50) and (B) fluorescence microscopy (magnification, x50). GFP, green fluorescent protein.

Ultrastructure examination of ovarian cancer cells.

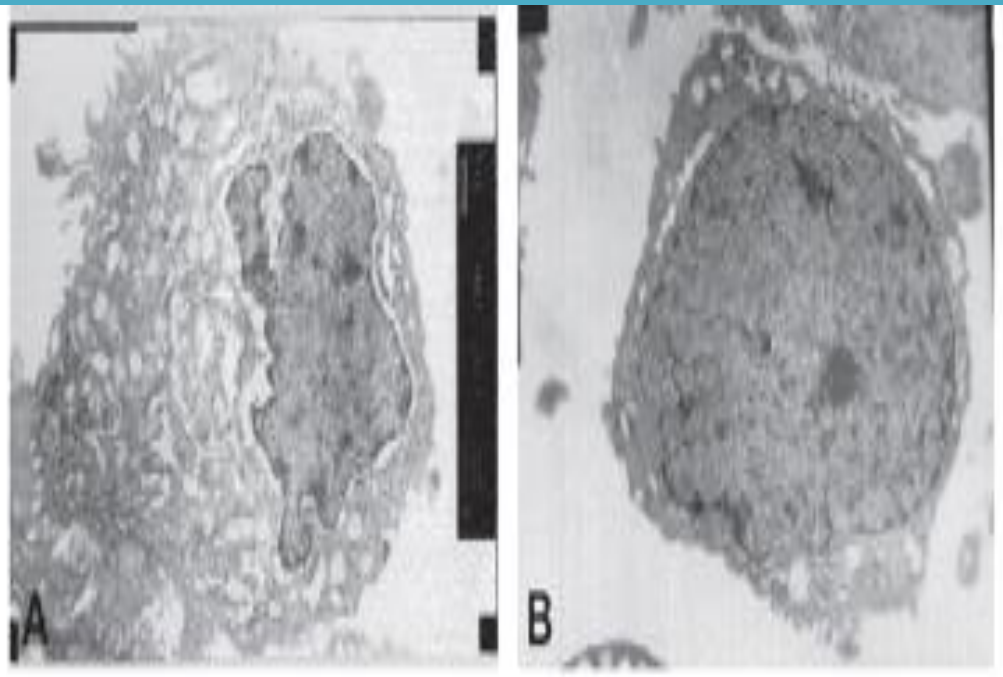

Figure 14. Transmission electron microscopic observation of apoptosis in SKOV3 cells (magnification, x4,000). (A) Apoptotic SKOV3 cell displaying a swollen endoplasmic reticulum and (B) SKOV3 cell from the control group (magnification, x4,000).

Discusstion

- Ovarian cancer is a common type of malignant tumor
- threatens the physical and mental he alth of females
- majority of patients reached an adva nced stage when they are diagnosed
- IL-2 administration stimulates T cell p roliferation[10]
- physicians attempted to use IL-2 to cure patients suffering with me tastasized ovarian cancer
- high concentrations of IL-2 are re quired
- MSCs systemic or local administr ation has been investigated in a v ariety of tumor animal models

- AF-MSCs may be considered as a po werful tool for gene therapy
- in the present study intravenousl y injected AF-MSCs, that stably e xpress hIL-2,

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- In the present study, AF-MSCs transd uced with GFP were analyzed in a m ouse ovarian cancer model to evaluat e the migratory properties in vivo
- are able to trace the subcutaneo usly transplanted ovarian tumor c ells, and secrete IL-2 locally, resu lting in the apoptosis of the tumor cells.

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